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MEDICAL VIROLOGY 10

Medical Virology 10

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FOREWORD

This year marks the tenth anniversary of the International Symposium on Medical Virology. In the Foreword to the book of the 1980 Symposium, we stated, "However, the challenges still lying ahead are more numerous than our past accomplishments". Little did we know at the time, that within a few years the spread of human immunodeficiency virus type I was going to occur. This worldwide epidemic has, like no other disease in recent history, awakened the scientific community and the public at large. It is a reminder to all of us that regardless of our vast technical advances, Nature provides such great opportunity for biological diversity, that it will always be one step ahead of our scientific knowledge. Although our understanding of virology, molecular biology and immunology have increased by leaps and bounds over the last decade, we are still at the point of being unable to effectively control the spread of this viral infection. We hope that our Symposium this year has helped researchers to come together and exchange ideas, so that our growing knowledge of viral infections will help produce better approaches to control them.

Luis M. de la Maza
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Irvine, California
March, 1991

ACKNOWLEDGEMENTS

It would be impossible to single out all those individuals who helped us make this Symposium a reality, however, we would like to take this opportunity to express our appreciation for their efforts. Special recognition should go to the speakers for their excellent lectures and chapters that contributed to this book. We also want to recognize Drs. Thomas C. Cesario and Edwin E. Lennette for chairing the sessions. The participation of the attendees in the discussions, poster sessions, and informal conversations provided an exciting, intellectual and scientific experience.

Special recognition should go to Marie Pezzlo and Sandra Aarnaes who, throughout the year, provided continued support to the organization of this meeting. We are very grateful to Penny Welter for her secretarial support and typing skills in preparing the camera-ready manuscripts for this book. The Plenum Publishing Company helped us with editorial support, and particular mention should be made of Melanie Yelity, Mary Safford and Gregory Safford for their help.

Finally, we want to express our appreciation to the following organizations who provided financial support, and helped to make the Symposium a reality:

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CHANGING TRENDS OF DIAGNOSTIC VIROLOGY IN A TERTIARY CARE MEDICAL CENTER

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INTRODUCTION

Mayo Clinic (MC) has 290,000 new patient registrations each year in a multidiscipline tertiary care practice located in a small community with a population of 75,000. Many of the patients are immunosuppressed such as those undergoing treatment for neoplastic or rheumatologic diseases. Others are admitted with acquired immunodeficiency syndrome (AIDS) or receive organ transplants (cornea, kidney, bone marrow, liver, pancreas, cardiothoracic) in a program that has involved 2,362 procedures. The increasing numbers of immunosuppressed patients, compared to 10-15 years ago, has had a profound effect on both the frequency and types of viruses recovered in the clinical laboratory. This communication compares the detection of viruses during a period from 1974-1982 with our experience in 1988.

METHODS

Cell Culture

Specimens collected with a Culturette™ (Becton-Dickinson, Cockeysville, MD) swab (respiratory, dermal, genital, ocular, gastrointestinal) were extracted into 2 ml of serum-free medium and inoculated into conventional tube and/or shell vials seeded with MRC-5 cells and into primary rhesus monkey kidney cell cultures. Tissue specimens were homogenized in a Stomacher Lab-Blender (Tekmar Co., Cincinnati, OH), centrifuged and the supernatant fraction inoculated into cell culture. Leukocytes from blood were separated by Ficoll/Paque-Macrodex (Pharmacia, Piscataway, NJ) or by Sepracell (Sepratech Corp., Oklahoma City, OK) prior to inoculation into cell cultures. Body fluids such as urine and cerebrospinal fluid (CSF) were inoculated directly into the two culture systems. Viral isolates were initially detected in tube cell cultures by cytopathic effects or by hemadsorption and identified by specific antibodies in immunofluorescence tests. Similarly, monoclonal antibodies to early viral antigens were used to rapidly detect viruses in shell vials (Gleaves et al. 1984; Smith, 1985).

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Serology

Serum specimens were assayed for IgG class antibodies using anticomplement (herpesviruses) or indirect (measles, mumps, RSV, influenza virus types A and B) immunofluorescence methods. An aliquot of the serum specimen was reacted with goat anti-human IgG (Whittaker MA Bioproducts, Walkersville, MD), incubated at room temperature for 30 min, and then centrifuged at 700 x g for 10 min. The supernatant fraction was tested for the presence of anti-CMV IgM (Smith & Shelley, 1986).

RESULTS

Specimens

Between 1974 (10,000) and 1990 (August, 66,312; projected 100,000), our laboratory has had a 10-fold increase in the numbers of specimens submitted (Figure 1). A substantial decrease in specimen counts occurred in 1984 (13%) associated with governmental reimbursement for medical services based on Diagnostic-Related Groups (DRG). Specimens referred by Mayo Medical Laboratories (MML) represented 65% (50,002) of our total workload; however, only 21% (11,122) of these samples had requests for viral detection.

Of almost 77,000 specimens submitted during 1988, 70% were requests for viral diagnosis (Table 1). Specimens received by our laboratory for the diagno-

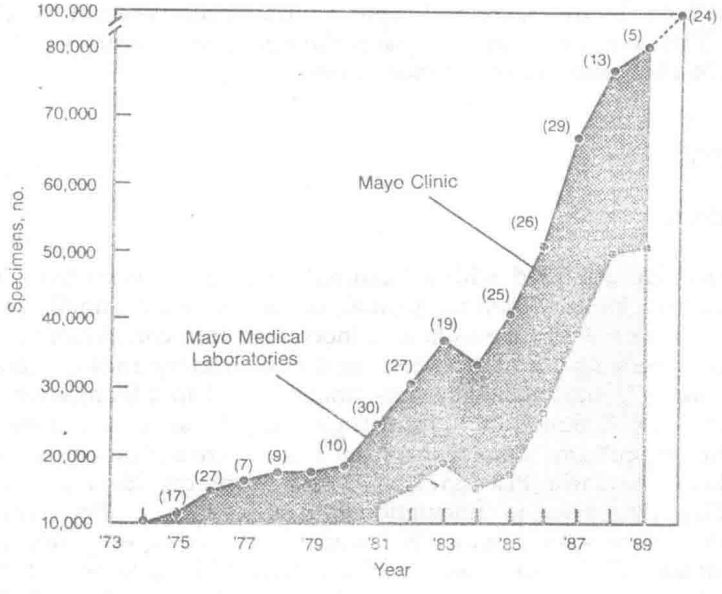

Figure 1. Specimens submitted to the laboratory for the diagnosis of viral, chlamydial and mycoplasmal infections. Total virology specimens, 1974-1990.

TABLE 1. Requests for Microbiology Testing Sent to the Virus Laboratory, Mayo Clinic, 1988

Microbiology Request	Culture	Serology	Total	% of Total Specimens
Virus	18,742	35,427[a]	54,169	70.3
C. trachomatis	2,149			
	6,443 (Micro Trak)	1,110	9,702	12.6
Mycoplasma/ ureaplasma	81	3,711	7,776	10.1
	3,984			
C. difficile	4,973		4,973	6.5
P. carinii	354 (Stain)		354	0.459
Total	36,726	40,248	76,974	100

[a] Includes screening tests for viral antibodies (VZV, EBV, rubella) and rickettsial requests (n = 13,802).

sis of *Chlamydia trachomatis* (13%), *Mycoplasma/Ureaplasma* (10%), *Clostridium difficile* toxin (6%), and *Pneumocystis carinii* (1%) collectively comprised 30% of the total. Serologic tests represented 51% (40,238) of the total assays (76,974) in 1988. Eighty-eight percent (35,427) of these requests were for viral agents, 9% for *M. pneumoniae* and 3% for *C. trachomatis*.

Comparison of Viruses Detected During Years 1974-1982 with 1988

Of 4,181 viruses detected during 1974-1982, 57% were herpesviruses, herpes simplex virus (HSV) 44%; cytomegalovirus (CMV) 7%; varicella-zoster virus (VZV) 7% (Figure 2). Enteroviruses (16%), recovered in the summertime, and influenza viruses (10%), isolated exclusively in the winter months, were next in frequency to HSV during this interim. In contrast in 1988, CMV was the most prevalent isolate (Figure 3). Altogether, the herpesviruses (CMV, 43%; HSV, 37%; and VZV, 3%, accounted for 83% of the isolates during that single year. Rotavirus antigen assay (Kallestad, Austin, TX), available as a routine test in 1985 in our laboratory, provided a diagnosis in 119 instances generally associated with pediatric gastroenteritis. Similarly, another rapid enzyme immunoassay (EIA) for antigen (Abbott Laboratories, Abbott Park, IL) was instituted two years later and provided a laboratory diagnosis of 82 cases of respiratory syncytial virus (RSV) infection. Therefore, utilizing rapid EIA (rotavirus, RSV) and the shell vial assay (CMV, HSV), our laboratory provided a diagnosis within 16 h postinoculation of the specimen for 88% of the viruses detected during 1988. Two other viruses, (VZV, 3%; adenovirus, 2%) were detected 48 h after inoculation into shell vials. Thus, rapid diagnostic techniques were in place for 93% of the 2,416 viruses detected that year.

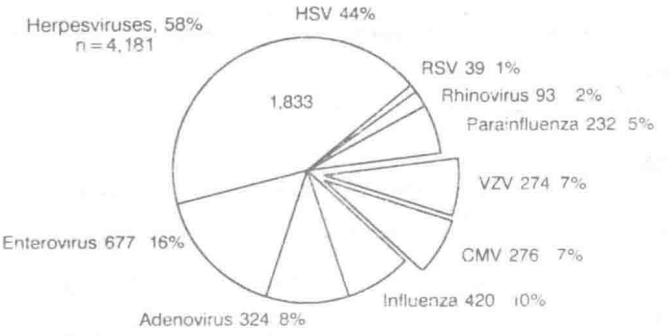

Figure 2. Detection of viruses, as percent of total. Mayo Clinic, 1974-1982.

Viruses Detected During 1988

MML accounted for 59% of the specimens (11,122/18,742) and 53% of the viruses (1,291) yielding a detection rate of 11.6% (Table 2). A higher rate of viral detection (14.8%) was obtained from specimens from local MC patients compared to the referral MML samples.

Respiratory and other (CSF and body fluids other than urine, tissues other than lung, eye, rectal) sources represented 48% of the specimens, 33% of the total viruses recovered, with an isolation rate of 10%. Conversely, dermal sites accounted for only 5% of the specimens, 11% of the viruses, but the highest yield of 30% compared to any other source (Figure 4).

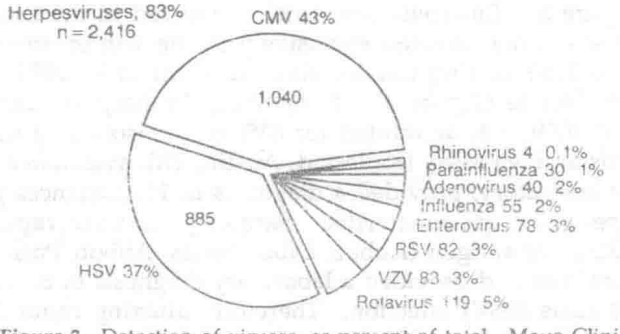

Figure 3. Detection of viruses, as percent of total. Mayo Clinic, 1988.

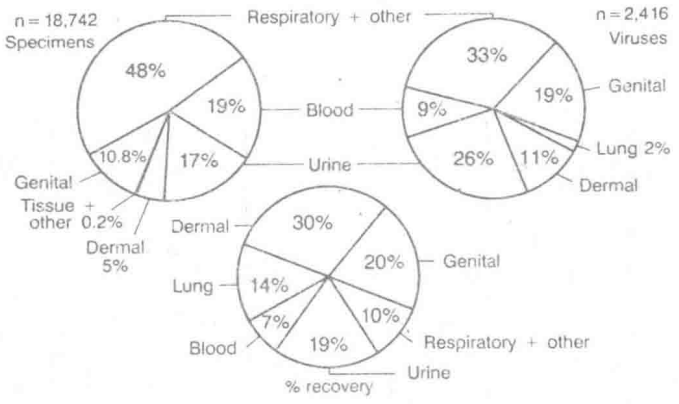

Figure 4. Specimens submitted, viruses detected, and percent recovery. Mayo Clinic, 1988.

Cytomegalovirus

Urine was the most productive source for the detection of CMV (608 isolates, 59% of the total detected) (Table 3). Importantly, among all viruses, CMV was the most predominant agent recovered from blood (219/225, 97%), bronchoalveolar lavage (BAL) (71/80, 89%), and tissue specimens (74/96, 77%) (Table 4).

Herpes Simplex Virus

As expected, genital sources provided over one-half of the total HSV isolates and more than 30% of these detected from this site were type 1 (Tables 5 and 6). HSV produced systemic disease occasionally as indicated by detection of the virus in brain, lung, and CSF. The single isolate from CSF was type 1 from a 3-year old with meningitis (Table 5). Interestingly, 113/190 (60%) of HSV isolates from dermal (nongenital) sources were type 2 (Table 6).

Rotavirus

Pediatric nursery and other outbreaks of gastroenteritis generally prompted clinicians to submit specimens for the diagnosis of rotavirus infection. Rotaviruses (119 detected) were the third most prevalent agent detected during 1988, indicating the need and importance of this test for routine use (Table 2).

Varicella Zoster Virus

VZV was recovered exclusively from dermal sites with the exception of one isolate from blood submitted through MML from a 33-year-old man (Table 7).

TABLE 2. Detection of Viruses form Specimens Submitted to the Mayo Clinic Virus Laboratory, 1988

Virus	Specimen																				% Total
	Genital				Urine				Dermal				Lung				Blood				Other[a]				Total				
	MML[b]		MC[c]		MML		MC		MML		MC		MML		MC		MML		MC		MML		MC		MML		MC		
CMV	0		0		166		442		0		0		30		10		39		180		104		69		339		701		43.0
																										1040			
HSV	380		68		1		4		140		50		1		0		0		0		142		99		664		221		36.6
																										885			
Rotavirus	0		0		0		0		0		0		0		0		0		0		93		26		93		26		5.0
																										119			
VZV	0		0		0		0		30		51		0		0		0		1		0		1		30		53		3.4
																										83			
RSV	0		0		0		0		0		0		0		0		0		0		30		52		30		52		3.4
																										82			
Enteroviruses	0		0		0		0		0		0		2		0		1		0		64		11		67		11		3.2
																										78			
Influenza virus	0		0		0		0		0		0		1		0		0		0		16		38		17		38		2.3
																										55			
Adenovirus	0		0		0		0		0		0		2		0		4		0		30		4		36		4		1.7
																										40			
Parainfluenza virus	0		0		0		0		0		0		0		0		0		0		12		18		12		18		1.2
																										30			
Rhinovirus	0		0		0		0		0		0		0		0		0		0		3		1		3	4	1		0.2
Total Viruses	380		68		167		446		170		101		36		10		44		181		494		319						
		448				613				271				46				225				813				2,416			100
Specimens	1,575		570		1,067		2,161		409		498		205		93		2,162		1,215		5,705		3,083		11,122		7,620		
		2,145				3,228				907				298				3,377				8,787				18,742			
% Virus Recovery	24.1		11.9		15.7		20.6		41.6		20.3		17.6		10.7		2.0		14.9		8.7		10.3						
		20.9				19.0				29.9				15.4				6.7				9.3			11.6%		14.8%		12.9
% Total Viruses		18.5				25.4				11.2				1.9				9.3				33.7							100

[a] Other: Respiratory (throat, sputum, bronchial wash and other secretions), CSF, and other body fluids (not urine), tissues other than lung, eye, and rectal.
[b] MML, Mayo Medical Laboratory
[c] MC, Mayo Clinic

TABLE 3. Detection of Cytomegalovirus, Mayo Clinic, 1988

Source of Specimen	No. Detected (%) MML	MC	Total	% From Source
Urine	166 (27)	444 (73)	610	59
Blood	39 (18)	180 (82)	219	21
BAL	28 (39)	43 (61)	71	6
Lung	30 (75)	10 (25)	40	4
Bronchial wash	38 (100)		38	4
Liver	4 (17)	19 (83)	23	2
Sputum	13 (100)		13	1
Throat	10 (100)		10	1
Colon	3 (50)	3 (50)	6	0.5
Nasal swab	4 (100)		4	0.5
Esophagus	1 (33)	2 (67)	3	0.3
Stomach	1 (50)	1 (50)	2	0.3
Eye	1 (100)		1	0.1
TOTAL	339 (33)	701 (67)	1,040	100

Ortho- and Paramyxoviruses

Respiratory syncytial virus. RSV, all from the respiratory tract (90% nasopharyngeal or nasal sources) comprised only 3.4% of the total viruses detected in 1988 (Tables 2 and 8). The low proportion of RSV cases (3.4%) likely reflects the predominant tertiary care nature of MC and the low population base of the immediate community for primary care medical services. Over 91% of the RSV cases occurred in children ≤2 years of age.

Influenza and Parainfluenza Viruses. The detection of influenza virus by specific monoclonal antibodies allowed rapid identification of strains according to serotype A or B (Table 8). The average age of patients from whom influenza virus was isolated from BAL or lung tissue was 62. These viruses accounted for 7/9 (78%) of the isolates from BAL that were not CMV (71/80, 89%) (Table 4).

Picornaviruses

Enteroviruses. Enteroviruses were identified on the basis of CPE only in MRC-5 and primary rhesus monkey kidney cells. Enterovirus was the most common viral cause of central nervous system (CNS) disease in children. Of 78

TABLE 4. Number of Viruses Recovered from BAL, Blood, CSF, Eye and Tissue Specimens, Mayo Clinic, 1988

Specimen	CMV	HSV	VZV	Entero	Influenza	Para-Influenza	Adeno	TOTAL
Blood	219		1	1			4	225
BAL	71			2	3	4		80
Eye	1	21					8	30
CSF		1		16			2	19
Tissue	74							
Lung	40	1		2	1		5	49
Liver	23						5	28
Colon	6	4						10
Esophagus	3							3
Stomach	2							2
Brain		1		1				2
Pericardium				1				1
Spleen				1				1
Total	**365**	**28**	**1**	**24**	**4**	**4**	**24**	**450**

TABLE 5. Detection of Herpes Simplex Virus, Mayo Clinic, 1988

Source of Specimen	No. Detected (%) MML	MC	Total	% From Source
Genital	380 (85)	68 (15)	448	51
Throat, sputum, mouth	116 (59)	80 (41)	196	22
Dermal	140 (74)	50 (26)	190	22
Eye	10 (48)	11 (52)	21	2
Rectal	7 (78)	2 (22)	9	1
Nose	5 (56)	4 (44)	9	1
Urine	1 (20)	4 (80)	5	0.4
Esophagus	2 (50)	2 (50)	4	0.3
CSF	1 (100)		1	0.1
Brain	1 (100)		1	0.1
Lung	1 (100)		1	0.1
TOTAL %	664 (75)	221 (25)	885	100

TABLE 6. Detection of Herpes Simplex Virus from Genital and Dermal Sites, Mayo Clinic, 1988

		Genital			
Source of Specimen	Total (%)	MML HSV-1 (%)	MML HSV-2 (%)	MC HSV-1 (%)	MC HSV-2 (%)
		N = 380		N = 68	
Genital	448 (70)	119 (31)	261 (69)	22 (32)	46 (68)
		N = 140		N = 50	
Dermal	190 (30)	49 (35)	91 (65)	28 (56)	22 (44)
TOTAL (%)	638	520 (82)		118 (18)	

TABLE 7. Detection of Varicella-Zoster Virus, Mayo Clinic, 1988

Source of Specimen	No. Detected (%) MML	MC	Total	% From Source
Dermal	30 (3.7)	52 (63)	82	99
Blood	1 (100)	0	1	1
TOTAL	31	52	83	100

TABLE 8. Detection of Ortho- and Paramyxovirus, Mayo Clinic, 1988

Source RSV	Source of Specimen	No. Detected (%) MML	MC	Total	% From Source
RSV	Respiratory tract[a]	30 (37)	52 (63)	82	100
Influenza	Respiratory tract	15, type A (30) 1, type B	35, type A (70)	51	91
	BAL		3	3	6
	Lung	1		1	3
				55	100
Para-influenza	Respiratory tract	12 (46)	14 (54)	26	87
	BAL		4	4	13
				30	

[a] Throat, nasopharyngeal, broncheal secretions